EXPERIMENTAL TYPE III PNEUMOCOCCUS PNEUMONIA IN MONKEYS

II. Treatment with an Enzyme Which Decomposes the Specific Capsular Polysaccharide of Pneumococcus Type III

By THOMAS FRANCIS, Jr., M.D., EDWARD E. TERRELL, M.D., RENÉ DUBOS, Ph.D., and OSWALD T. AVERY, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research*)

Plates 48 to 50

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In the preceding paper (1) the production and the clinical features of experimental Type III pneumococcus pneumonia in monkeys of the *M. cynomolgos* species were described. The experimental disease in its clinical aspects closely resembles lobar pneumonia in man. The infection in these animals results in a pneumonic lesion of lobar distribution which tends to spread and which terminates after a variable period in spontaneous recovery or death. The irregularity of the course of the disease in individual animals is striking, but when septicemia is present the mortality rate increases proportionately with the number of organisms in the circulating blood. Thus, the height of the septicemia, irrespective of the size of the infecting dose, was found to be the most valuable objective index of the severity of the disease in a given animal.

In earlier studies, Avery and Dubos (2) showed that a specific enzyme of bacterial origin was capable of protecting mice against subsequent infection with Type III Pneumococcus, and of exerting a curative effect on infections already established. The beneficial effect of the enzyme was demonstrated to be due to its capacity to decompose the specific capsular polysaccharide of Type III Pneumococcus, thus rendering the bacteria readily susceptible to phagocytosis by the cells of the animal body.

Studying the effect of the enzyme upon a dermal infection with Type III Pneumococcus in rabbits, Goodner, Dubos, and Avery (3)

found that 95 per cent of untreated rabbits died, while of those which were treated with enzyme, 95 per cent recovered. With the amounts of enzyme employed, there was found to be a degree of infection which, although influenced by enzyme, terminated fatally. Goodner and Dubos (4) later showed that the amount of enzyme required for successful therapy increased with the height of the septicemia.

Since it had been found possible regularly to produce an experimental pneumonia in monkeys very similar to lobar pneumonia in man, it was of interest to determine the effect of treatment with specific enzyme upon the course and outcome of the disease. It was fully recognized that the methods employed in the production of enzyme were not standardized, and that different lots of enzyme varied considerably in their potency. Certain lots even contained substances which were toxic for animals. In spite of these technical imperfections, the treatment of experimental lobar pneumonia in monkeys was begun. The present paper reports the results of enzyme treatment of experimental Type III pneumococcus pneumonia in 40 monkeys. All animals were treated within the first 3 days after infection. Consequently, they have been divided into groups on the basis of the height of the septicemia during the first 3 days of the disease. This classification affords a basis for comparison of the results with the 68 untreated monkeys included in the preceding paper.

EXPERIMENTAL

Selection of Animals for Treatment.—Because of the extreme variations in the severity of the experimental disease in different monkeys, it was not feasible to select alternate animals for treatment and controls. Only after the disease was established was it possible to determine which animals in a given experiment were the most likely to succumb. Consequently, the sickest animals in each experimental group (2 to 6 in number) were chosen for enzyme therapy. The criteria on which the choice was based were the fever, the number of circulating leukocytes, the X-ray evidence, the general condition of the animal, and especially the height of the septicemia.

Enzyme.—The preparations of the enzyme were made by methods previously described (5); but from time to time minor changes were introduced in attempts to produce a more potent product. The enzyme content of the different preparations varied from 2 to 20 units per cc.

Procedure of Treatment.—Practically all therapeutic experiments were carried out during the winter and spring, when experimental conditions were more nearly

stable and the smaller doses of organisms were more efficient in producing typical pneumonia. During the period of treatment the animal's temperature was recorded, repeated blood cultures and blood counts were made, and the X-rays were carefully studied for evidence of extension or regression of the pneumonic process. A blood culture and white blood cell count were made immediately before treatment. Because of variation in the enzyme content of different lots, the usual procedure was to administer 10 cc. of the enzyme preparation intravenously. Further treatment was based upon the response to the first injection, the severity of the disease, and the potency of the particular preparation of enzyme. Additional treatments were given as often as three times daily, either intravenously, intraperitoneally, or by both routes simultaneously. When enzyme therapy was employed, treatment was always begun within the first 3 days after infection.

TABLE I

*Mortality in Experimental Type III Pneumococcus Pneumonia in Monkeys Receiving Enzyme Therapy**

Diagnosis	No. of animals	No. recovered	No. died	Mortality
				per cent
Pneumonia without septicemia	8	8	0	0
Pneumonia with septicemia (1–250 per cc.)	15	15	0	0
Pneumonia with septicemia (250–2000 per cc.)	9	8	1	11.1
Pneumonia with septicemia (2000 or greater per cc.)	8	1	7	87.5
Total	40	32	8	20.0

* Classified on the basis of height of septicemia during first 3 days.

No attempt was made to ascertain the minimal amount of enzyme required in individual monkeys, but, rather, treatment was intensively employed until the result was assured.

RESULTS

In the entire series of 40 monkeys with pneumonia, in which enzyme therapy was employed, the mortality rate was 20 per cent. The animals have been subdivided into four groups based on the height of the septicemia during the first 3 days of the disease (Table I). The distribution of the cases is comparable to that of the untreated series (1). With one exception, the fatal cases fall into the group with extreme septicemia and marked prostration. The height of the septi-

cemia during the first 3 days, and the number of pulmonary lobes involved, in relation to recovery or death, are represented graphically in Chart 1. The amount of pulmonary involvement was, in general, greater in the fatal cases than in those which recovered. The salient features of the disease in the different groups are discussed in detail.

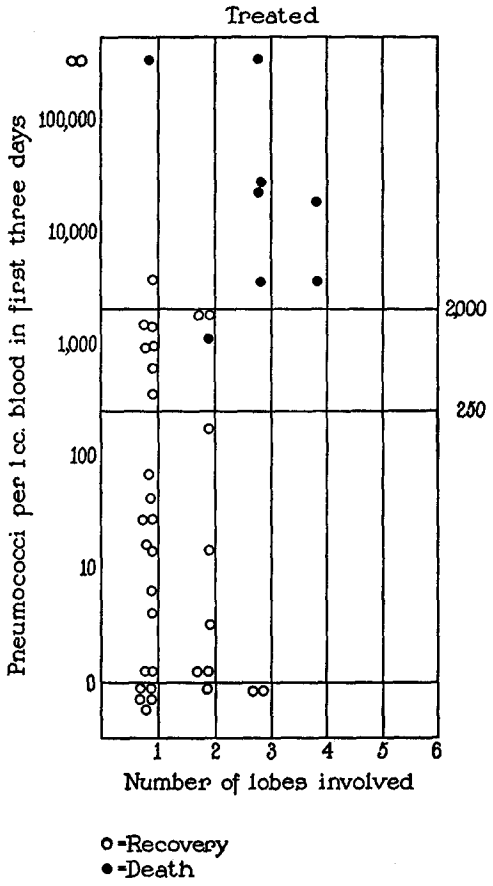

CHART 1. The relation of the height of septicemia and the amount of pulmonary involvement to the outcome of the disease.

Group A. Lobar Pneumonia without Septicemia

In this group of 8 animals, no demonstrable invasion of the blood had occurred at the time treatment was begun, which in 6 instances

was on the 1st day, in 2 on the 2nd day after infection. All 8 animals recovered. The average weight of the group was 2130 gm. The average amount of culture employed for inoculation was 0.35 cc. In 2 cases there was involvement of three pulmonary lobes, in 1 case two lobes, while in the remaining 5 only one lobe was involved. The average duration of the disease was 3.2 days after infection. This was estimated, to a great extent, upon the time when regression of the pneumonia was noted in the X-ray. The temperature curve and the white blood count were of less value, since some febrile reaction and fall in the number of circulating leukocytes frequently followed administration of enzyme. When a rise in the number of leukocytes began, however, recovery was usually definitely established.

No attempt was made to ascertain the minimal amount of enzyme required. The number of units of enzyme given to animals in this group varied from 100 units to 360, and the number of treatments varied from a single dose to four over a period of 3 days. In most instances, improvement began shortly after treatment was instituted. The monkey became more alert; there was a tendency for the fever to subside; regression of the shadow, most noticeable at the margins, was observed in the X-ray film.

In comparison with the untreated animals (1) without septicemia, the course of the disease in the treated animals was, on the average, of shorter duration. Furthermore, from a clinical standpoint, the animals of this group were, at the time treatment was begun, sicker than those of the untreated group, and the amount of pulmonary involvement was greater. In some instances, it is possible that with a spreading pneumonia, septicemia and death might have occurred in the absence of treatment. In no instance was a prolonged illness noted, although one monkey, No. 4-2, while recovering from pneumonia, developed cutaneous sores and on the 6th day a septicemia due to *Staphylococcus aureus* and a Gram-negative bacillus. When sacrificed, the lungs showed definite resolution and the cultures from the pneumonic lobes revealed no pneumococci.

Monkey 1-11 (Chart 2) was treated on the 2nd day after infection, when the right upper lobe was completely involved. At that time the temperature was high, but following a single treatment with 50 units of enzyme there was a critical fall of the fever; no spread of the lesion occurred. On the 3rd day another treat-

TABLE II

Experimental Type III Pneumococcus Pneumonia in Monkeys Receiving Enzyme Therapy

No	Weight	Dose	Route*	Date		Days after infection 1	2	3	4	5	6	7	Result†	Remarks
						Pneumonia without septicemia								
	gm.	*cc.*												
3-3	1650	0.4	i.t.	4–11–32	Bl. cult.‡	0 0	0	0	0	0			R 2	
					WBC 17.7§	12.6, 8.1	6.9	8.1	16.6	20.4				
					X-ray‖	1/3 RUL 1/3 RML 1/3 RLL	Clearing	Clearing	Clearing	Clearing				
					Enzyme units	49	35, 35	17.5						
4-0	2150	0.4	i.t.	5–10–32	Bl. cult.	0 0	0	0	0				R 3	
					WBC 23.0	12.8	3.5	6.1	7.5					
					X-ray	1/2 RLL 1/2 RML 1/2 RUL	Spread	Clearing	Clearing		Clearing			
					Enzyme units	120, 120	120							
4-2	2350	0.4	i.t.	5–10–32	Bl. cult.	0 0	0	0	0		Staph., Gram-neg. bacillus	Staph., Gram-neg. bacillus	R 4	Sacrificed 9th day. Many sores over body
					WBC 15.4	5.9	1.1	1.8	1.7		5.5	1.7		Pneumonia RLL (resolving); RML, same; RUL, same
					X-ray	1/2 RLL	RLL 1/2 RML	RLL RML	Clearing		Clearing			HB culture: Staph. and Gram-neg. bacillus
					Enzyme units	120, 120	120							Culture: RLL negative
7-6	2150	0.25	i.b.	12–12–32	Bl. cult.	0	0	0	0	0			R 3	
					WBC 21.5	24.0	12.5	8.0	3.6	5.1				
					X-ray	1/3 RLL	3/4 RLL	Clearing	Clearing	Clearing				
					Enzyme units		60	60						
7-8	2150	0.25	i.b.	12–12–32	Bl. cult.	0	0	0	0	0			R 3	
					WBC 18.8	23.8	12.5	10.5	20.6	14.1				
					X-ray	1/2 RLL	Same	Clearing	Clearing	Clearing				
					Enzyme units	80	30							

8-1	2900	0.25	i.b.	12–19–32	Bl. cult.	0	0	0	0				R 4	
					WBC 17.0	54.2	31.4	15.1	11.5	28.6				
					X-ray	1/2 RUL	Spread	RUL	Clearing	Clearing				
					Enzyme units	60	85	85						
1-11	1950	0.4	i.b.	5–15–33	Bl. cult.	0	0	0	0				R 4	Sacrificed 5th day
					WBC 18.1	28.3	18.2	12.8	12.6					RUL consolidated throughout, resolving in lower portion
					X-ray	2/3 RUL	RUL	RUL RML?	Clearing	Clearing				Cultures: RUL negative
					Enzyme units		50	50						
1-19	1750	0.45	i.b.	5–29–33	Bl. cult.	0	0	0	0				R 3	Sacrificed 16th day
					WBC 14.6	20.8	20.2	15.1	13.7					Upper 1/2 RLL yellow and flabby suggesting advanced resolution
					X-ray	1/3 RLL	1/2 RLL	Clearing	Clearing					
					Enzyme units	100								
Pneumonia with septicemia, 1–250 colonies per cc. in first 3 days after infection														
2-9	1600	0.3	i.t.	3–14–32	Bl. cult.	14, 0	0	0	0	0	0		R 3	
					WBC 20.3	20.0	5.2	8.5	15.3	19.8		18.9		
					X-ray	RML 1/2 RLL	Denser	Clearing	Clearing	Clearing				
					Enzyme units	40	20	20						
3-0	2200	0.3	i.t.	3–14–32	Bl. cult.	30, 0	0	0	0	0	0		R 3	
					WBC 14.8	12.0	4.0	9.1	9.0	21.6				
					X-ray	1/2 RLL	Same	Clearing	Clearing	Clearing				
					Enzyme units	40	20	20						
3-4	1600	0.4	i.t.	4–14–32	Bl. cult.	0, +	0	0	0				R 2	
					WBC 18.4	12.7	7.2	5.6	7.8					
					X-ray	1/2 RLL	Clearing	Clearing	Clearing	Clearing				
					Enzyme units	42	42, 42	21						

* i.t. = intratracheal inoculation; i.b. = intrabronchial inoculation.

† R = recovery. D = death. Numerals indicate the day of recovery or death. F. D. = found dead.

‡ Number of pneumococci obtained in poured plate culture per 1 cc. of blood; + = growth occurred in broth cultures of blood.

§ White blood cells in thousands per c. mm. of blood.

‖ RUL, RML, RLL, LUL, LML, LLL = right upper, right middle, right lower, left upper, left middle, left lower lobes, respectively. (Cardiac lobe not included.)

TABLE II—*Continued*

No.	Weight	Dose	Route*	Date		Days after infection 1	2	3	4	5	6	7	Result†	Remarks
Pneumonia with septicemia, 1–250 colonies per cc. in first 3 days after infection—*Concluded*														
	gm.	*cc.*												
6-2	2350	0.35	i.b.	11–1–32	Bl. cult.	5	2, 0	0	0				R 4	
					WBC 11.4	22.4	10.7	11.7	9.6					
					X-ray	1/2 RLL	Spread	RLL RML	Same		Clearing			
					Enzyme units		80	80						
7-7	2450	0.25	i.b.	12–12–32	Bl. cult.	+	0	0	0				R 4	
					WBC 18.9	23.8	19.2	11.6	17.3					
					X-ray	1/3 RLL	Denser	Spread	Clearing	Clearing				
					Enzyme units		60							
8-4	1950	0.25	i.b.	1–4–33	Bl. cult.	50	70	0	0	0			R 4	
					WBC 18.1	22.4	18.0	17.1	11.3	20.6				
					X-ray	1/3 RLL	1/2 RLL	Spread		Clearing	Clearing			
					Enzyme units		200	200						
8-6	2200	0.3	i.b.	1–11–33	Bl. cult.	0	+	0	Gram-neg. bacillus	Gram-neg. bacillus			R 3	Maintained septicemia of Gram-neg. bacillus. Developed swollen, contractured legs. Died 15 days later. Lungs clear
					WBC 22.5	7.6	11.8	7.8	16.0	27.2				Cultures: HB Gram-neg. bacillus. RLL negative
					X-ray	RUL 1/3 RLL	RUL 1/2 RLL	Clearing	Clearing	Clearing				
					Enzyme units		180	200						
8-8	2300	0.3	i.b.	1–16–33	Bl. cult.	0	3	8	0	0			R 5	
					WBC 31.9	24.4	14.6	20.0	8.5					
					X-ray	1/3 RUL	Spread	2/3 RUL	2/3 RUL	Clearing				
					Enzyme units			200						
9-2	2050	0.3	i.b.	1–30–33	Bl. cult.	10	16	0	0	0			R 4	
					WBC 19.7	13.3	8.4	6.8	8.6	21.4				
					X-ray	1/2 RLL	RLL	RLL	Clearing	Clearing				
					Enzyme units		200	180						

9-4	2100	0.33	i.b.	2–6–33	Bl. cult.	41	13	14, 18	1	4	0	0	R 7	On the 4th, 5th, and 6th days a Gram-neg. bacillus was also found in blood stream. Enzyme contaminated
					WBC 22.7	19.0	15.7	4.6	10.4	14.4	18.2			
					X-ray	2/3 RUL	RUL	Denser	Clearing	Clearing				
					Enzyme units		200	120, 130, 120	120					
1-18	1900	0.4	i.b.	5–15–33	Bl. cult.	166	60	0, 0	0				R 3	
					WBC 26.0	42.8	18.0	9.2, 6	12.8					
					X-ray	RML	RML 1/3 RUL	Clearing	Clearing	Clearing				
					Enzyme units		50	50, 50						
1-14	1600	0.4	i.b.	5–15–33	Bl. cult.	14	0	0	0				R 3	Compare with No. 1-1, preceding paper
					WBC 13.9	30.5	13.6	11.3	8.0					
					X-ray	RML	Same	Clearing	Clearing	Clearing				
					Enzyme units		50							
1-20	1900	0.45	i.b.	5–23–33	Bl. cult.	6	5	0	0				R 3	
					WBC 19.9	23.0	11.4	6.9	9.7					
					X-ray	RML	RML	Clearing	Clearing					
					Enzyme units		90	90						
1-23	1700	0.45	i.b.	5–23–33	Bl. cult.	6	43	0	0				R 4	
					WBC 21.4	25.2	15.2	15.0	20.4					
					X-ray	2/3 RLL	RLL	1/2 RUL RLL	Clearing					
					Enzyme units		90	90						
1-24	1750	0.45	i.b.	5–29–33	Bl. cult.	0	1	+	0				R 5	
					WBC 21.1	34.0	19.4	22.6	10.2					
					X-ray	1/2 RUL	Denser	2/3 RUL	RUL	Clearing				
					Enzyme units			100	55					
Pneumonia with septicemia, 250–2000 colonies per cc. in first 3 days after infection														
3-8	2260	0.4	i.t.	4–18–32	Bl. cult.	3	344, 2	0	0	0		0	R 4	
					WBC 15.6	29.0	24.0, 16.0	10.3	17.4	16.5		36.3		
					X-ray	1/2 RLL	3/4 RUL	Denser	Clearing	Clearing		Clearing		
					Enzyme units		67, 45	40	15					
8-0	1900	0.25	i.b.	12–19–32	Bl. cult.	43	360	1700	400	404	516	6	R 8	*Blood culture on 8th day sterile*
					WBC 18.3	12.6	8.4	1.4	1.0	30.2	37.0	29.0		
					X-ray	1/3 RLL	2/3 RLL	RLL	RLL RML	Clearing		Clearing		
					Enzyme units		85	85	170		170			

TABLE II—*Continued*

No.	Weight	Dose	Route*	Date		Days after infection 1	2	3	4	5	6	7	Result†	Remarks
Pneumonia with septicemia, 250–2000 colonies per cc. in first 3 days after infection—*Concluded*														
	gm.	*cc.*												
8-7	2250	0.3	i.b.	1–11–33	Bl. cult.	1	414	995	0	4	0	0	R 6	
					WBC 14.9	14.3	9.6	5.7	4.9	7.2	16.0	12.0		
					X-ray	1/2 RLL	2/3 RLL	RLL	Same	Clearing	Same	Clearing		
					Enzyme units			360	258		200			
9-5	2075	0.33	i.b.	2–6–33	Bl. cult.	536	1600	1728	141	157	15	0	R 7	
					WBC 21.0	16.2	8.5	8.7	14.6	22.5		44.0		
					X-ray	RUL	RUL 1/3 RML	Same	Clearing	Clearing	Clearing			
					Enzyme units	190	200	174, 126, 120	132					
7-2	1275	0.33	i.b.	2–27–33	Bl. cult.	1400, 4	19	0	0	0			R 4	
					WBC 14.7	4.2	3.5	4.4	6.0	15.7				
					X-ray	1/2 RUL	Spread	Spread	Clearing	Same		Clearing		
					Enzyme units	130, 130	130, 117	143, 156						
1-26	1700	0.45	i.b.	5–29–33	Bl. cult.	616	408, 4	65	0	0			R 4	
					WBC 10.8	16.1	12.6	4.9	11.3	15.6				
					X-ray	1/2 RML	Spread	RML	Clearing	Clearing				
					Enzyme units		200, 100	100	90					
1-29	1950	0.5	i.b.	6–5–33	Bl. cult.	148	1480	4, 174	6, 0	0			R 4	
					WBC 10.6	21.0	18.5	3.8	16.6	26.6				
					X-ray	2/3 RUL	Spread	RUL	Clearing			Cleared		
					Enzyme units		55	55, 150	150, 120					
1-31	1650	0.5	i.b.	6–5–33	Bl. cult.	175	744	904, 190	336, 226	20		0	R 6	Sacrificed 8th day
					WBC 15.4	14.0	8.2	4.8	11.6	24.0		34.0		Lower 2/3 RLL resolving pneumonia
					X-ray	1/2 RLL	RLL	RLL	Clearing			Clearing		
					Enzyme units		55	55, 150	150, 120	120				

4-3	1875	0.4	i.t.	5–10–32	Bl. cult. WBC 30.0 X-ray Enzyme units	1040, 160 2.6, 1.1 1/2 RLL 1/3 RUL 120							D 2	Animal died suddenly as if choking Autopsy: Pericarditis; pneumonia: 1/2 RLL, 1/3 RUL
Pneumonia with septicemia greater than 2000 colonies per cc. in first 3 days after infection														
2-1	1275	1.0	i.t.	2–8–32	Bl. cult. WBC 17.5 X-ray Enzyme units	4800 3.8 LLL	124,000 7,800 2.7 LLL LML 2/3 LUL 130						D 2	Autopsy: Fibrinopurulent pleurisy; pericarditis; pneumonia LLL, LML, 2/3 LUL. *B. lactis aerogenes* also present in cultures at autopsy
2-4	1200	0.5	i.t.	2–11–32	Bl. cult. WBC 12.2 X-ray Enzyme units	24,000 7.2 2/3 LLL 1/3 RLL 156							D 1	Autopsy: Empyema, left; pneumonia LLL, 1/2 LML, 1/3 RLL, Culture: HB–Pn. III, 1520 colonies per cc.
3-1	1725	0.3	i.t.	3–21–32	Bl. cult. WBC 19.9 X-ray Enzyme units	18,000, 0 7.0, 1.5 1/2 LLL LUL 25	+ 3.3 LLL LML LUL 25, 25	3200 6.5 Same 25, 12.5	4800 Same 25	2000 20.1 Spread RML			D 5	Autopsy: Pneumonia LLL LML, LUL, RML
3-9	3025	0.4	i.t.	4–18–32	Bl. cult. WBC 23.9 X-ray Enzyme units	2800 13.2 1/2 LLL	25,000 3,750 7.0, 1.3 3/4 LLL 90, 60, 60	700 2.2 Same 40, 30	3750 6.2 Same 35				D 4	Autopsy: Fibrinopurulent pleurisy; pericarditis; pneumonia LLL; congestion LML, RLL
9-1	1900	0.3	i.b.	1–23–33	Bl. cult. WBC 23.9 X-ray Enzyme units	6500 20.0 1/2 RLL	∞ 9.5 Same 400						F. D. 3	Autopsy: Empyema, right; pneumonia 1/3 RLL; congestion RML, RUL

TABLE II—*Concluded*

No.	Weight	Dose	Route*	Date		Days after infection 1	2	3	4	5	6	7	Result†	Remarks
Pneumonia with septicemia greater than 2000 colonies per cc. in first 3 days after infection—*Concluded*														
9-8	2050	0.33	i.b.	2–27–33	Bl. cult.	3520	2680						F. D. 4	Autopsy: Pericarditis; pneumonia RUL, RML, 1/2 RLL, LML
					WBC 18.3	4.8	2.0							
					X-ray	RUL	RUL							
						1/2 RML	RML							
						LML	LML							
					Enzyme units	130, 130	130 143							
1-17	1600	0.4	i.b.	5–15–33	Bl. cult.	137	2292	3520					D 3	Autopsy: Pneumonia RUL, RML, 1/3 RLL; fibrinopurulent pleurisy
					WBC 13.2	18.1	20.5	13.3						
					X-ray	1/2 RUL	RUL	RUL						
							RML	RML						
								1/3 RLL						
					Enzyme units		50	50, 50						
1-30	1725	0.5	i.b.	6–5–33	Bl. cult.	33	3800	52, 1000	1056, 188	0			R 5	
					WBC 10.1	18.0	7.9	5.0	5.6	10.3		23.6		
					X-ray	1/3 RUL	1/2 RUL	Clearing	Clearing	Clearing				
					Enzyme units		55	110, 150	150, 150					

ment of the same amount was given. On the 4th day resolution was evident in the X-ray. Recovery progressed uneventfully.

Monkey 8-1 (Chart 3). Treatment was begun on the 1st day of the disease, but extension of the pneumonia was noted in the X-rays on the 2nd and 3rd days. On the 4th day resolution was evident. Roentgenograms taken during the course of the disease and recovery are reproduced in Figs. 1 to 6.

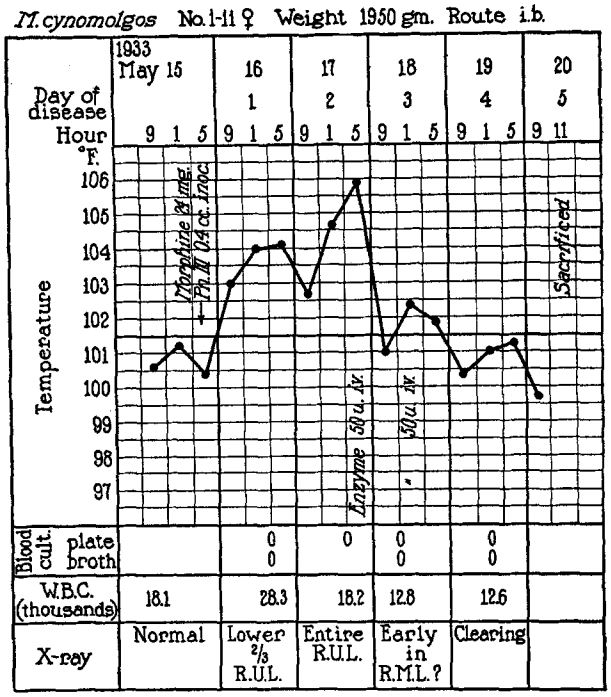

CHART 2. Experimental pneumonia without septicemia, treated with enzyme. *u.* represents units of enzyme.

Group B. Lobar Pneumonia with Septicemia (1–250 Colonies per Cc.)

In this group of 15 animals the average weight was 1880 gm., the average amount of culture used was 0.37 cc. In 5 cases, two pulmonary lobes were involved, in the remainder only one lobe. Septicemia was present in all instances, the number of organisms present in the blood at the time of treatment ranging up to 166 colonies per 1 cc. of blood in the first 3 days after infection. 3 animals were

treated on the 1st day, 10 on the 2nd, and 2 on the 3rd day after infection. The amount and duration of treatment varied from a single dose of 50 units on the 2nd day, to 5 treatments over the 2nd, 3rd, and 4th days, with a total of 692 units. In one case, No. 1-14, the blood culture contained 14 colonies per cc. on the 1st day, but was sterile on the 2nd day, when treatment was begun. The only other example

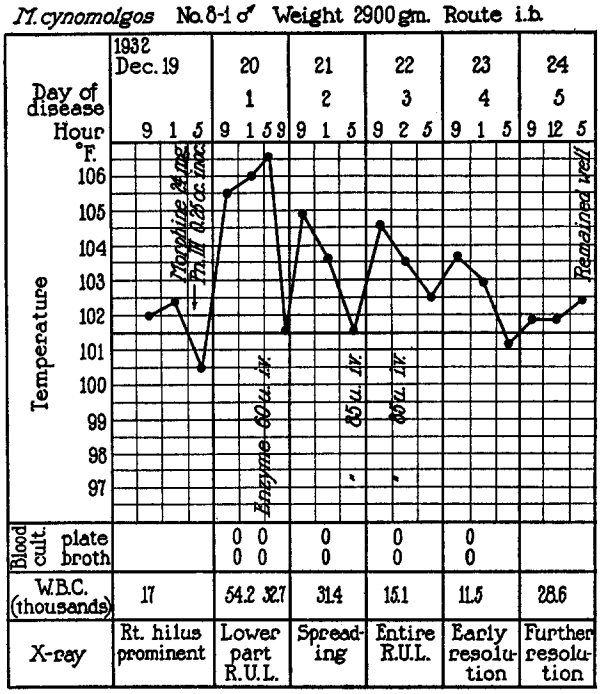

CHART 3. Experimental pneumonia treated on 1st day of disease.

of this course of events occurred in Monkey 1-1 of the untreated group, in which septicemia recurred and a fatal termination resulted. In all but 2 cases the pneumonic process was clearly extending at the time of treatment.

Treatment of this group of animals resulted in 100 per cent recovery, in contrast to 55 per cent recovery in the untreated group with comparable septicemia. The average time of recovery was 3.6 days after infection. In 13 of the 15 cases, sterilization of the blood was effected

by the first treatment, in some instances within 4 hours. In one exception, the enzyme was found later to be contaminated, and Type III Pneumococcus was recovered in culture from the blood together with a Gram-negative bacillus. In spite of this, resolution began promptly and continued. In the other case, the blood culture at the time of the first treatment was negative, but a second culture taken $2\frac{1}{2}$ hours later was positive. A third culture on the following day was negative. In another animal, following therapy and while recovery was under way, an intercurrent superficial infection of the extremities was noted, and a Gram-negative bacillus was isolated from the blood.

In 10, or possibly 11 cases, no spread of the pneumonic lesion was demonstrable by X-ray the day following the first treatment; in the majority, resolution was clearly seen. In the 4 cases in which extension of the lesion was noted the 1st day after treatment, there was no further progression as treatment was continued. Concomitantly with the improvement as shown by sterilization of the blood and decrease in the size of the pneumonic process, there was usually a fall in temperature, except in cases in which febrile reactions followed later injections of enzyme. The animals also became stronger and more alert.

In comparison with similar untreated cases (1), the duration of disease in the treated animals was, on the average, almost 1 day shorter than in the untreated animals which recovered, and 2 days shorter than in the fatal cases. Of the untreated cases which recovered, only 2 of 11 presented septicemia on the 1st day of disease, whereas of the 9 fatal cases, 7 had positive blood cultures on the 1st day. The treated cases appear to be more comparable to the latter animals, since of the 15 treated animals 13 had positive blood cultures the 1st day after infection, and, in general, higher septicemias than the fatal cases of the parallel untreated group. The pulmonary involvement was comparable in that progressively spreading pneumonia of lobar distribution was present at the time of treatment. The prompt subsidence of the septicemia and limitation of spread after treatment, together with general improvement, contrasts sharply with the course of the disease in the untreated series.

Monkey 1-18 (Chart 4) represents the type of case in which two pulmonary lobes were involved early in the disease and a relatively high septicemia (60

colonies per cc.) was present. Treatment begun on the 2nd day resulted in prompt control of the pneumonia, and recovery of the animal.

Monkey 9-2 (Chart 5) illustrates the type of case in which treatment was begun on the 2nd day. The blood, which contained 16 colonies per cc. at the time of treatment, was promptly sterilized, but the X-ray the following day showed some extension of the pneumonia. There is the possibility that the spread

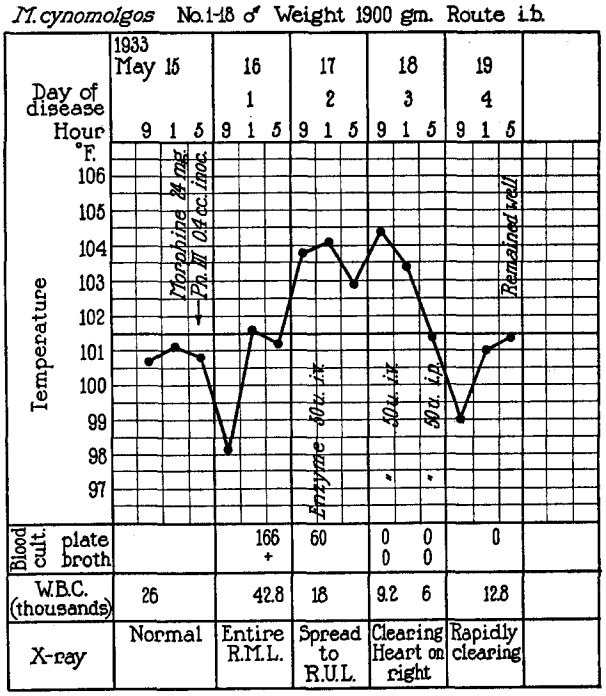

CHART 4. Effect of enzyme on experimental pneumonia with mild septicemia.

of the lesion seen in the X-ray on the 3rd day occurred in the interval of 6 hours between the time of the X-ray and the time treatment was begun. Nevertheless, recovery rapidly followed.

Group C. Lobar Pneumonia with Septicemia (250–2000 Colonies per Cc.)

This group comprises 9 animals in which the experimentally induced pneumonia was accompanied by a high septicemia, ranging from the mildest case with 344 colonies per cc. of blood in the first 3 days after infection, to 2 cases with 1700 and 1728 colonies, respectively, during

that period. Needless to say, the degree of illness in this group was severe. However, 8 of the treated animals recovered, giving a recovery rate of 88.9 per cent. This contrasts with the results in untreated animals with similar severity of disease of which only 25 per cent recovered.

The average weight of the animals was 1880 gm.; the average amount of culture injected was 0.39 cc. In the 1 animal which died, there was involvement of two

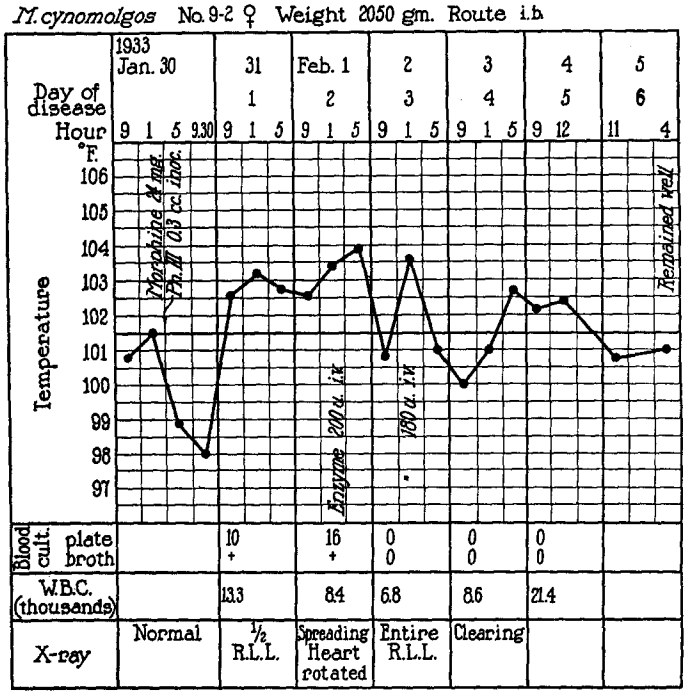

CHART 5. Experimental pneumonia with mild septicemia treated with enzyme.

lobes the morning after infection, and a blood culture yielded 1040 colonies per 1 cc. of blood. A treatment of 120 units of enzyme was given 4½ hours later. 6 hours after the treatment, the septicemia was reduced to 160 colonies per cc. At this time, the monkey had an attack of choking and died. A search at autopsy revealed no tracheal obstruction, but definite pneumonic consolidation was present in the right upper and lower lobes, and Type III Pneumococcus was recovered from the pericardial fluid. The spleen was small and bound by dense adhesions.

In 2 of the 8 recovered cases, two lobes were involved; in the remaining 6, only one lobe was affected. In 6, the leukocyte count dropped below 5000 at the height of the disease, and in one case to 1000, but at the beginning of recovery a definite rise occurred. 5 were treated on the 2nd day after infection, 2 on the 1st day, and one on the 3rd day. Repeated treatments were given, the total amounts of enzyme varying from 177.5 units to 942 units. Although in 3 instances the degree of septicemia increased after treatment was instituted, in only 2 was there definite extension of the pneumonia after treatment. In fact, in 4 cases the

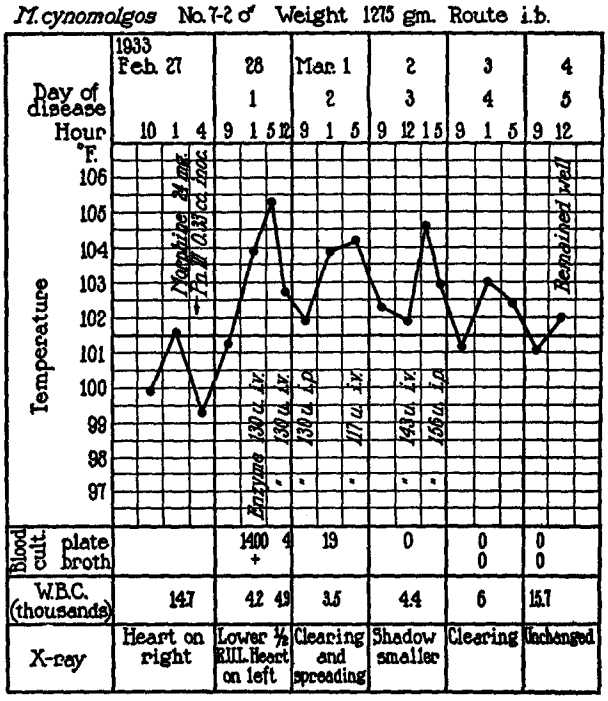

CHART 6. Effect of enzyme on experimental pneumonia with moderately severe septicemia.

roentgenograms revealed the beginning of resolution before the blood was cleared of organisms. Furthermore, a fall in temperature and an improvement in the general condition of the animals occurred at about the same time regression of the lesion was noted by X-ray, even though the septicemia might continue for several days (Monkey 8-0). In general, in spite of the high septicemia, the action of the enzyme in effecting the sterilization of the blood was usually prompt and striking; the average time of recovery, based on final sterilization of the blood and X-ray evidence, was 5.4 days after infection.

To evaluate the effect of enzyme treatment in these animals, they may be compared with the untreated (1) group of monkeys with comparable pneumonia and septicemia.

In 5 of the 9 fatal cases in the untreated group, the pneumonic process spread to involve three or more lobes. Extension of the pneu-

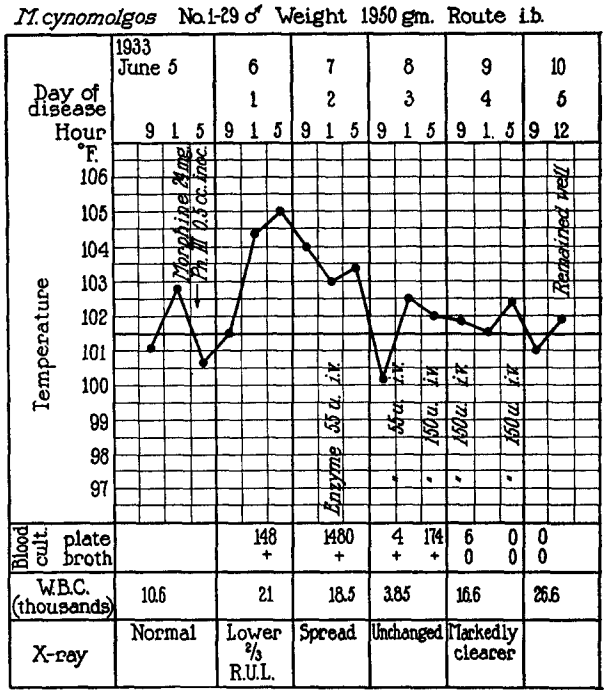

CHART 7. Experimental pneumonia with moderately severe septicemia treated on 2nd day of disease.

monia was greatly limited in the treated group. In 6 of the untreated cases, empyema, pericarditis, or both, were found at autopsy; under treatment, these complications were either prevented, or, if present, (and the incidence in the untreated would suggest the probability) they also responded favorably to treatment. In the treated group, the degree of the septicemia at the time of treatment was consistently more severe than it was in the untreated animals at comparable times.

The results in this group of animals leave little doubt of the value of enzyme therapy in experimental Type III pneumococcus pneumonia.

Monkey 7-2 (Chart 6) showed pneumonic consolidation of the mid-portion of the right upper lobe on the 1st day after infection. A blood culture yielded 1400 colonies of Type III Pneumococcus per cc. of blood, and the leukocytes had dropped to 4200 per c.mm. The first treatment, given 24 hours after infection, resulted in a prompt drop in the septicemia. Four treatments in the next 48 hours brought about prompt recovery. Even on the 2nd day, when the lesion was spreading in part of the lobe, the original site of consolidation was clearing.

Monkey 1-29 (Chart 7) is an instance in which treatment was begun on the 2nd day after infection when most of the right upper lobe was consolidated and the septicemia had reached a height of 1480 colonies per cc. Four treatments during the next 2 days resulted in obvious resolution of the pneumonia, sterilization of the blood, and recovery.

Group D. Lobar Pneumonia with Septicemia (More than 2000 Colonies per Cc.)

This group of 8 monkeys represents the extreme form of infection in which the tendency was toward a diffuse pulmonary involvement, overwhelming septicemia, extreme fall in white blood count, failure in febrile response, and early death. Nevertheless, of the 8 treated cases, 1 recovered, a mortality rate of 87.5 per cent.

The average weight of the animals was 1820 gm., varying from 1200 to 3025 gm. The average amount of inoculum was 0.46 cc. of Type III pneumococcus culture. In the fatal cases the average duration of the disease was 3 days. In 2 of the group, only one pulmonary lobe was involved; in the others three or more lobes were affected. In 6 of the 8 cases, Pneumococcus III was recovered from the pleural or pericardial fluid, or both. In 3 cases the septicemia was below 4000 colonies per 1 cc. of blood, in the remainder it ranged from 18,000 to innumerable colonies at the time treatment was begun. In inverse ratio to the degree of septicemia, the circulating leukocytes were severely depressed to 2000 or less per c.mm. The animals rapidly collapsed, and any therapeutic aid seemed hopeless.

The amount of enzyme administered varied from 130 units in 1 animal to 615 units in the recovered case. In 3 instances, only one dose of enzyme was given, and in 3 others repeated doses of relatively small amounts were given. In the latter animals, distinct effects were noted, and possibly with larger amounts of enzyme recoveries might have resulted.

Despite the forbidding aspects of the disease, one animal with involvement

of the right upper lobe and a septicemia of 3800 colonies at the time of the first treatment on the 2nd day recovered. In 2 cases with 18,000 and 25,000 colonies at the time treatment was begun, the septicemia was markedly reduced, the spread of the pneumonia checked, and life prolonged until the 5th and 4th days, respectively. In 2 others, although death occurred early, single treatments caused a marked diminution in the number of pneumococci in the circulating blood.

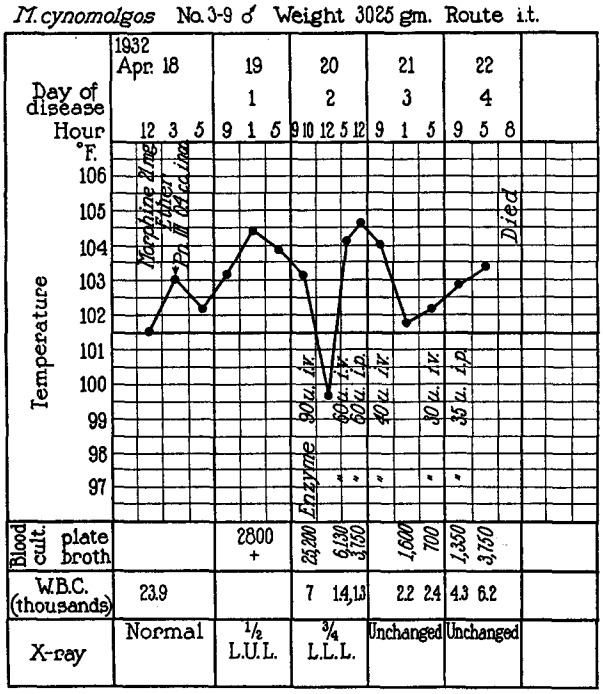

CHART 8. Effect of enzyme therapy in promoting the cessation of pneumonic extension, diminution of septicemia, and prolongation of life in experimental pneumonia with overwhelming septicemia.

The disease in this group of animals is much the same as that in the untreated (1) group with comparable septicemia, in which the mortality rate was 100 per cent. In comparing the degree of involvement in the two groups, the treated animals had, in a higher percentage, three or more lobes involved. However, animals suffering from pneumonia of such pronounced form are, because of the marked

general depression and rapidly fatal outcome, clearly unsatisfactory for therapeutic experiments.

Monkey 3-9 (Chart 8) illustrates the effect of enzyme therapy in a case with marked septicemia which terminated fatally. The spread of the pneumonia was checked, a substantial reduction in septicemia was effected, and life prolonged.

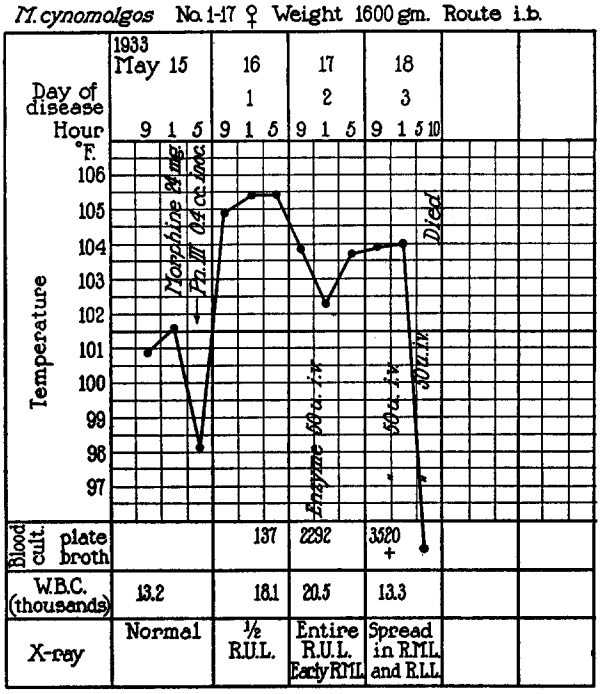

CHART 9. Experimental pneumonia with marked septicemia, treated with enzyme but terminating fatally.

At autopsy, the left lower lobe was completely consolidated, while the left middle and right lower lobes appeared congested. Fibrinopurulent pleurisy and pericarditis were present.

Monkey 1-17 (Chart 9) is an instance in which the pneumonia continued to spread, the septicemia increased, and death occurred on the 3rd day after infection. Autopsy revealed lobar pneumonia of the right upper and middle lobes, and partial consolidation of the right lower lobe. A fibrinopurulent pleurisy was present over the involved lobes.

In Monkey 1-30 (Chart 10), which had well marked consolidation at the time of treatment and 3800 colonies of Type III Pneumococcus in culture per 1 cc. of blood, treatment caused prompt limitation of the pneumonia, and two days later sterilization of the blood. Recovery followed. Roentgenograms are shown in Figs. 13 to 18.

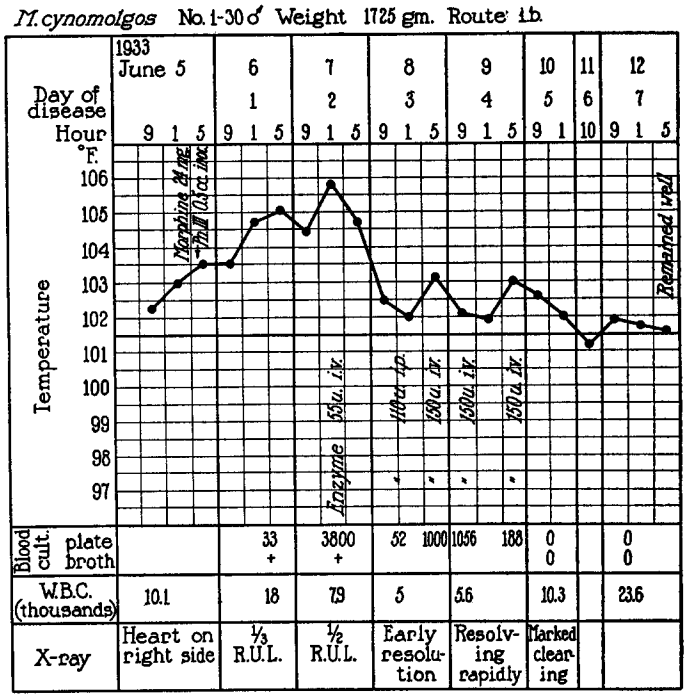

CHART 10. Experimental pneumonia with marked septicemia which recovered after enzyme therapy.

DISCUSSION

The results of specific enzyme therapy in the experimental Type III pneumococcus pneumonia of monkeys have been presented. To evaluate the effects of treatment in the present series of animals, the results, as measured by recovery or death, may be compared with those in an untreated series of animals (1) included in the preceding paper (Table III). It must be borne in mind, however, that in a given experiment the animals which appeared sickest were selected for treatment. Of those animals, treated or not, in which no bac-

terial invasion of the blood was demonstrable, all recovered. In the group with pneumonia accompanied by septicemia of 1 to 250 colonies per cc. of blood, 45 per cent of the 20 untreated animals died, while all of the 15 treated monkeys recovered. Of the 12 untreated animals in the group with septicemia ranging from 250 to 2000 colonies per cc., 75 per cent died, whereas of 9 animals which received enzyme therapy, only 1 died, a mortality rate of 11.1 per cent. The final group includes the animals which suffered from an extremely pronounced form of infection, frequently with diffuse pneumonia, rapid exhaustion of

TABLE III

*The Influence of Enzyme Therapy upon Mortality Rate in Experimental Type III Pneumococcus Pneumonia in Monkeys**

Class of infection	Untreated				Treated			
	No. of animals	No. recovered	No. died	Mortality	No. of animals	No. recovered	No. died	Mortality
				per cent				*per cent*
Pneumonia without septicemia	20	20	0	0	8	8	0	0
Pneumonia with septicemia (1–250)	20	11	9	45.0 } 56.2	15	15	0	0 } 4
Pneumonia with septicemia (250–2000)	12	3	9	75.0	9	8	1	11.1
Pneumonia with septicemia (2000+)	16	0	16	100.0	8	1	7	87.5
Total	68	34	34	50.0	40	32	8	20.0
Total for groups with septicemia	48	14	34	70.8	32	24	8	25.0

* Classified on the basis of height of septicemia in first 3 days.

circulating leukocytes, septicemia of very high degree, and with a tendency to early death. In the 16 untreated animals of this group, the mortality rate was 100 per cent. Of 8 comparable monkeys to which enzyme was administered, 7 died, and 1, in which the septicemia reached 3800 colonies per cc. before treatment, recovered, a mortality rate of 87.5 per cent.

It can readily be seen that in the groups in which no invasion of the blood occurs, spontaneous recovery is to be uniformly expected, whereas in the extremely severe forms of the disease the great majority of animals are too completely prostrated to respond to any therapeutic

aids. Consequently, the two intermediate groups appear to offer the best opportunity for studying the effects of enzyme therapy. Included in these groups in which septicemia ranged from 1 to 2000 colonies per cc. are 32 untreated monkeys of which 18 died (56.2 per cent), and 24 treated animals with only one death (4 per cent). Or, if one compares the results in all animals in which septicemia was present, 70.8 per cent of 48 untreated animals died, but only 25 per cent of the 32 treated animals.

In addition to the apparently beneficial effects of specific enzyme therapy as measured by survival or death of the animals, certain other favorable influences were observed. In a high percentage of cases in which extension of the pneumonic process was occurring at the time of treatment, the spreading promptly ceased following the initial injection of enzyme. Although the density of the area of consolidation might at first appear greater than before treatment, extension did not occur and resolution of the lesion soon began. This limitation of spread of the pneumonia was not infrequently noted in the severe cases before the bacteria were completely eliminated from the blood stream (Figs. 7 to 12). A comparison of the ultimate degree of pulmonary involvement in the treated and untreated cases (1) (Chart 1) reveals the fact that it was less, in general, in the former series. While in the treated cases the extension was apparently limited early, in the untreated animals extension of the pneumonia progressed, frequently with fatal results.

That the administration of enzyme promoted sterilization of the blood stream seems certain. In the milder cases this occurred quite rapidly. In animals in which the higher degrees of septicemia were present, there was rarely an increase, more regularly a prompt decrease in the number of pneumococci in the blood following the administration of enzyme. Even in cases which eventually terminated fatally, or in which extreme septicemia occurred early in the disease, cultures of the blood showed a marked reduction in the number of bacteria within 4 to 5 hours after the first treatment.

Simultaneously with limitation of the pneumonia, beginning resolution, and elimination of septicemia, a fall in temperature usually occurred. In fact, there was a tendency for the fever to subside concurrently with the cessation of pneumonic spread, even though

septicemia still persisted. Although a marked leukopenia was comparatively frequent at the time treatment was begun, the number of leukocytes rose with the beginning of recovery.

In fatal untreated cases with septicemia, a high incidence of positive cultures was obtained from pleural or pericardial fluids at autopsy. In many instances frank empyema or pericarditis was present. In the treated cases with severe infections which resulted fatally, the incidence of these complications was also high. In recovered animals of the treated series, therefore, a frequency of suppurative complications equal to that of the untreated animals might be expected. The fact that the treated animals which survived recovered without suppurative sequelae suggests that enzyme therapy either prevented the development of empyema and pericarditis or was therapeutically effective even in the presence of these complications.

As previously stated, many technical difficulties have been encountered in attempting to produce enzyme preparations of uniformly high therapeutic activity and purity. The different lots of enzyme have, as a result, been inconstant in both these respects. In some instances toxic effects, attributable to impurities in the material, have been noted in animals after the administration of enzyme. These impurities may induce a febrile reaction and a decrease in the white blood count of the animal. At other times, when the animal is extremely ill with subnormal temperature and a marked leukopenia, the administration of impure preparations may produce a further depression of temperature and of the leukocytes.

The results of the present study indicate that the specific enzyme, even in its present state of purity, exerts a favorable therapeutic effect upon the course and outcome of experimental Type III pneumococcus pneumonia in monkeys. Nevertheless, the present study again emphasizes the therapeutic limitations of the enzyme (4). The action of the enzyme is known to be exerted upon the capsular polysaccharide of Type III Pneumococcus (6). By being deprived of its capsule, the bacterium is made susceptible to phagocytosis by the cells of the animal body. However, when the disease process is of extreme severity and the entire cellular mechanism of the body is markedly depressed, the animal may no longer possess the capacity to dispose of the organisms rendered vulnerable by the specific action of the enzyme.

SUMMARY

The effects of specific enzyme therapy upon experimental Type III pneumococcus pneumonia in monkeys were studied by comparing the course and outcome of the disease in treated animals with that in animals which received no therapeutic aid. Enzyme treatment was found to exert a distinctly favorable influence upon the experimental pneumonia. Treatment was followed by cessation of spread of the pneumonic lesion, sterilization of the blood, and early recovery, except in animals in which the severity of the disease was extreme. While in the untreated animals a high incidence of empyema and pericarditis was observed, suppurative sequelae were apparently prevented by adequate enzyme therapy. The limitations of the therapeutic action of the specific enzyme in the presence of marked depression of the cellular reaction in infected animals are again emphasized.

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EXPLANATION OF PLATES

PLATE 48

Roentgenograms of Monkey 8-1 during the course of experimental pneumonia, treated the 1st day after infection (Chart 3).

FIG. 1. Control. Before inoculation (Dec. 19).

FIG. 2. (Dec. 20.) 19 hours after infection and 7 hours before the first treatment, showing well localized consolidation in lower half of the right upper lobe.

FIG. 3. 2nd day (Dec. 21), showing extension of pneumonia throughout right upper lobe.

FIG. 4. 3rd day (Dec. 22), showing increased density of the shadow over the right upper lobe, but no evidence of further spread.

FIG. 5. 5th day (Dec. 24). Resolution of the pneumonia has begun, as shown by the decrease in density and beginning aeration of the area.

FIG. 6. 10th day (Dec. 29), showing complete resolution of the pneumonic shadow.

PLATE 49

Roentgenograms of Monkey 9-4 during the course of the disease and recovery (Table II).

FIG. 7. 1st day after infection (Feb. 7), showing a well marked early pneumonia of the lower part of the right upper lobe.

FIG. 8. 2nd day (Feb. 8), showing extension of the lesion through the entire right upper lobe, and a small early shadow in the right cardiohepatic angle. Treatment begun.

FIG. 9. 3rd day (Feb. 9). Shows no extension, perhaps some clearing of shadow in right cardiohepatic angle.

FIG. 10. 4th day (Feb. 10). Aeration beginning, as evidenced by the clearly outlined base of heart.

FIG. 11. 5th day (Feb. 11). Base of heart drawn well to right toward resolving lobe, a feature not infrequently noted.

FIG. 12. 1 week later (Feb. 18). Almost complete resolution.

PLATE 50

Roentgenograms of Monkey 1-30, first treated on the 2nd day after infection when septicemia had reached 3800 colonies per cc. (Chart 10).

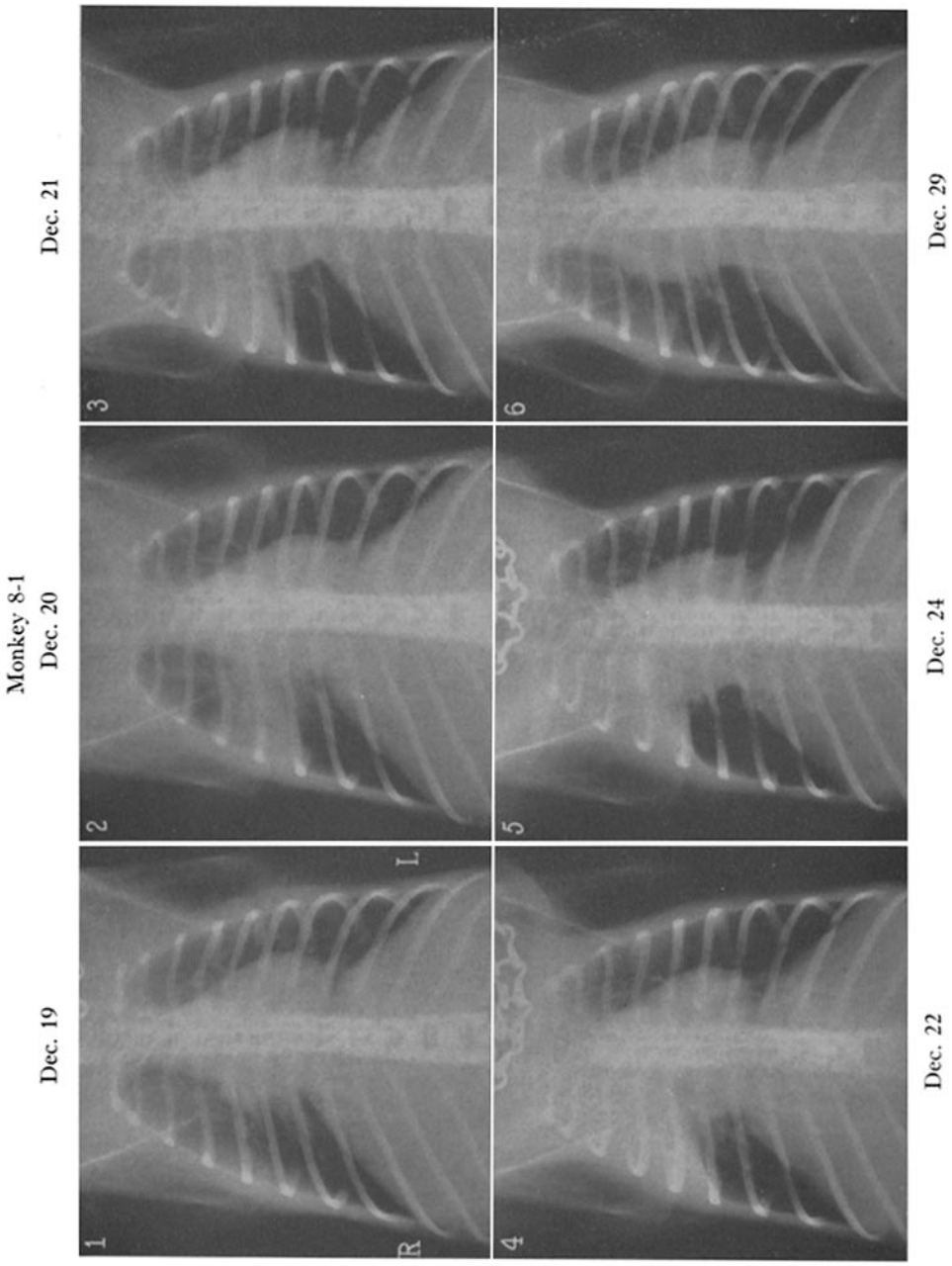
FIG. 13. Control plate (June 5). Shows heart rotated to the right side. Evidence of the extreme mobility of the mediastinum often noted.

FIG. 14. 1st day of disease (June 6), showing early lesion in the lower part of the right upper lobe.

FIG. 15. 2nd day (June 7), showing extension of lesion.

FIG. 16. 3rd day (June 8, following treatment). There is a decrease in density of the shadow, suggesting early resolution.

FIGS. 17, 18. 4th and 5th days (June 9 and 10), showing regression of the lesion.



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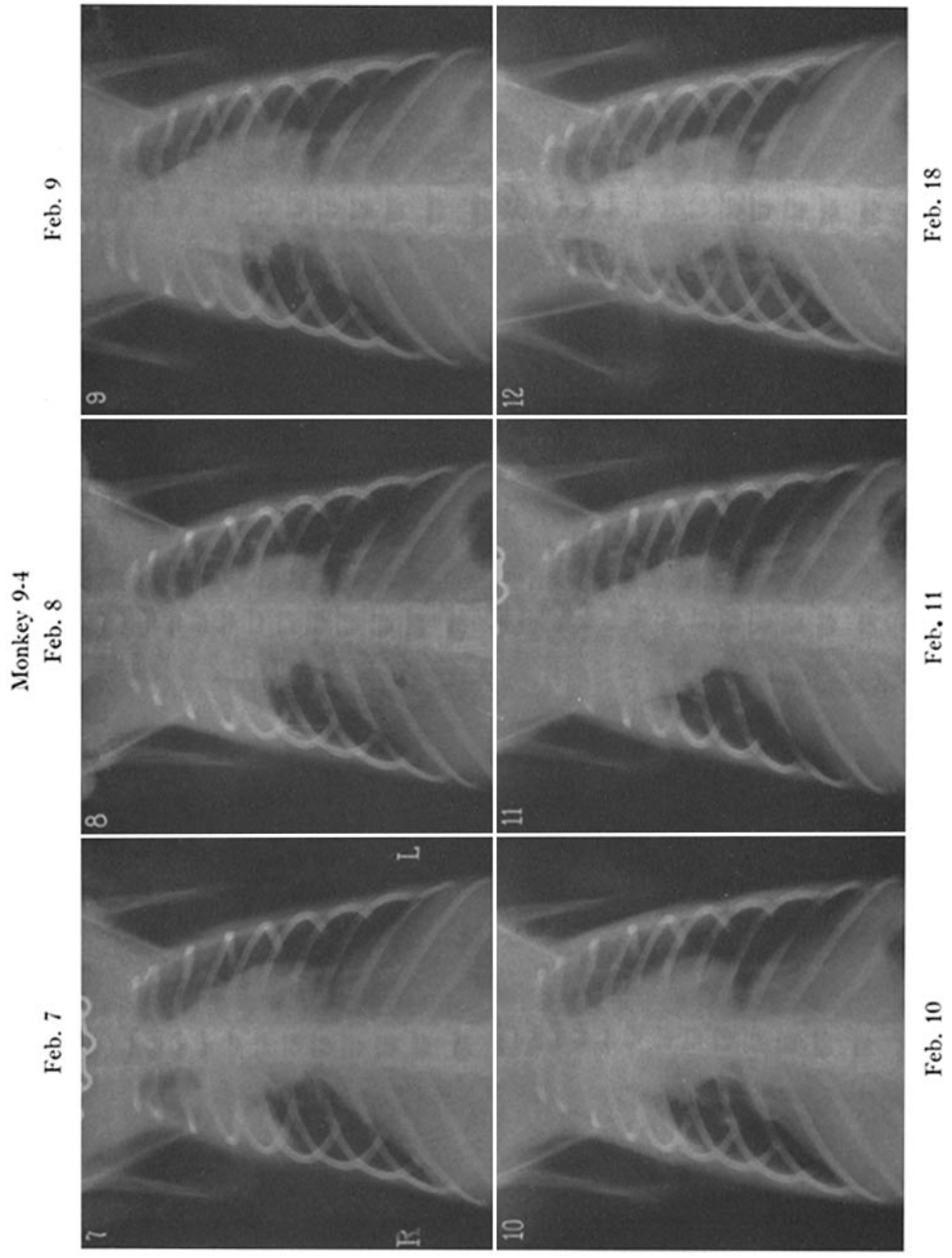

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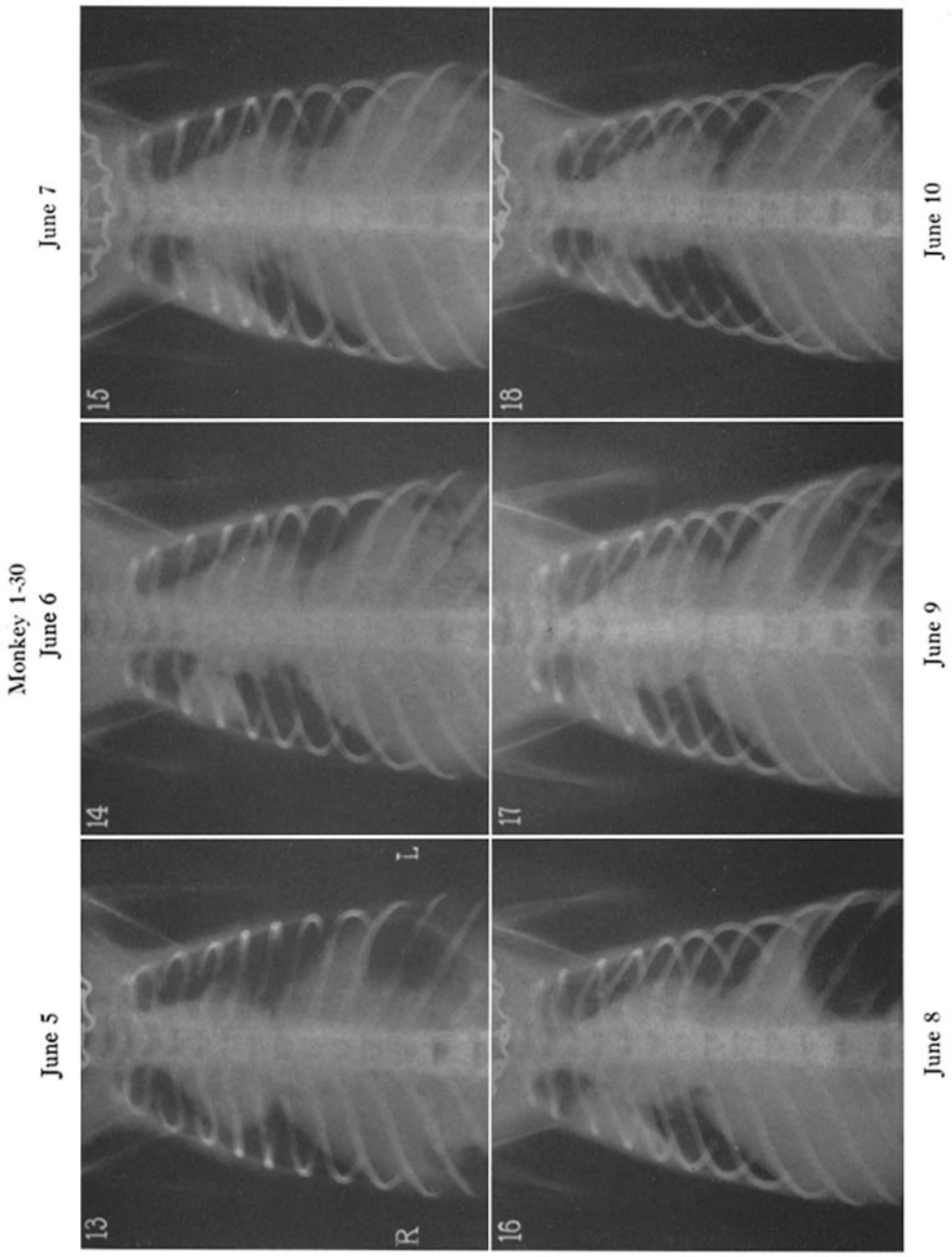

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